RESEARCH PAPER

Evaluation Of Antifungal Properties Towards Oral Candida Among Various Fruit Extracts Native to Dakshina Kannada District- An In-Vitro Study

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ABSTRACT

Background: Oral candidiasis is a common opportunistic infection caused by Candida albicans, particularly in immunocompromised individuals. Increasing antifungal resistance and limitations of conventional drugs such as Nystatin have prompted interest in natural alternatives. This study evaluated the antifungal efficacy of selected fruit extracts native to Dakshina Kannada district.

Aim: To evaluate and compare the antifungal activity of pulp extracts of Citrus sinensis, Artocarpus heterophyllus, Ananas comosus, Syzygium aqueum, and Borassus flabellifer against Candida albicans.

Materials and Methods: This in vitro experimental study assessed antifungal activity using broth microdilution for Minimum Inhibitory Concentration (MIC) and agar well diffusion for Zone of Inhibition (ZOI). Nystatin was used as a positive control and DMSO as a negative control. Statistical analysis was performed using independent t-test, one-way ANOVA, and Tukey's post-hoc test with significance set at $p < 0.05$.

Results: All fruit extracts demonstrated identical MIC values of 12.50%, whereas Nystatin showed a significantly lower MIC of 0.10% ($p < 0.001$). No significant difference was observed among fruit extracts for MIC ($p = 1.00$). In ZOI analysis, significant differences were noted among extracts at 50% concentration ($p < 0.001$), with Borassus flabellifer and Citrus sinensis showing the highest activity. At 6.25%, only Borassus flabellifer retained antifungal activity. Nystatin exhibited significantly greater inhibition than all extracts across all concentrations ($p < 0.001$).

Conclusion: While all fruit extracts exhibited antifungal properties, Borassus flabellifer showed comparatively superior activity, especially at lower concentrations. However, Nystatin remains significantly more potent, highlighting the need for further research on plant-based antifungal agents.

Keywords: Antifungal Efficacy, Nystatin,Fruit Extract, Zone of Inhibition, Minimum Inhibitory Concentration, Candidiasis

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INTRODUCTION

Candidiasis is a common opportunistic fungal infection primarily caused by Candida albicans, a commensal organism found on mucosal surfaces of the oral cavity, gastrointestinal tract, and genitourinary system. Under conditions of immunosuppression, prolonged antibiotic use, or poor oral hygiene, Candida can overgrow, leading to oral candidiasis, commonly known as oral

thrush [1]. Oral candidiasis presents with symptoms such as white patches, erythema, discomfort, and burning sensations, significantly affecting the patient's quality of life. The global prevalence of oral candidiasis varies, with higher rates in immunocompromised individuals, such as those with HIV/AIDS, diabetes, and elderly denture wearers, reaching up to 60% in some populations [2]. Furthermore, recurrent and chronic infections pose a growing clinical concern, particularly with rising antifungal resistance.

Nystatin, a polyene antifungal, remains the standard drug for treating oral candidiasis due to its fungicidal action by binding to ergosterol in fungal cell membranes, causing increased permeability and cell death [3]. However, nystatin has limitations, including poor palatability, gastrointestinal disturbances, and the risk of developing antifungal resistance with prolonged usage [4]. These drawbacks have driven interest in exploring alternative therapeutic agents, particularly those derived from natural sources with minimal side effects and additional health benefits [5].

Plant-based therapies have gained attention for their safety profiles, bioactive compounds, and potential antimicrobial properties. In regions like Dakshina Kannada, several native fruits are not only part of the traditional diet but are also valued for their nutritional and therapeutic benefits. Orange pulp (Citrus sinensis), often discarded as waste, is rich in vitamin C, flavonoids, and essential oils, contributing to antioxidant and anti-inflammatory properties that support oral and systemic health [6]. Jackfruit pulp (Artocarpus heterophyllus) is a nutrient-dense food containing vitamins A and C, dietary fiber, and polyphenols, which aid digestion and enhance immune function [7]. Pineapple (Ananas comosus) is well known for its enzyme bromelain, which possesses anti-inflammatory and digestive benefits, alongside high vitamin C content [8]. Watery rose apple (Syzygium aqueum) provides hydration and essential nutrients like vitamins A and C, making it a refreshing and health-supportive fruit with traditional use in promoting digestion and cooling the body [9]. Ice apple (Borassus flabellifer), consumed widely during summer months, is appreciated for its hydrating effects and electrolyte content, which help maintain fluid balance and prevent dehydration [10].

By evaluating these fruits extract in an in vitro model, this study aims to explore their potential as natural alternatives in managing oral Candida infections, aligning with the global need for safe, effective, and accessible antifungal strategies. This study aims to evaluate the antifungal efficacy of these plant extracts against C. albicans through in vitro analysis. By investigating the potential of natural antifungal alternatives, this research seeks to contribute to the development of safer, more effective, and sustainable treatment options for Candida infections, addressing the growing concern of antifungal resistance and treatment limitations associated with conventional drugs.

The study was conducted to evaluate and compare the antifungal efficacy of pulp extracts of Citrus sinensis, Artocarpus heterophyllus, Ananas comosus, Syzygium aqueum, and Borassus flabellifer against Candida albicans using minimum inhibitory concentration (MIC) and zone of inhibition (ZOI) tests.

MATERIALS AND METHODS

Study Design

This study was conducted as an in-vitro experimental investigation to evaluate the antifungal activity of selected fruit extracts native to the Dakshina Kannada district against Candida albicans. Antifungal efficacy was assessed using the agar well diffusion method for determination of the Zone of Inhibition (ZOI) and the broth microdilution method for estimation of the Minimum Inhibitory Concentration (MIC).

Ethical Approval

Ethical clearance for the study was obtained from the Institutional Ethics Committee prior to the commencement of the study.

Sample Size Calculation

The sample size was calculated using the formula:

$$n = \frac{2 \times SD^2 \times (Z_{\alpha/2} + Z_{\beta})^2}{d^2}$$

Where:

SD = 5.4

Zα/2 = 1.96 (at 5% type I error)

Zβ = 0.842 (at 80% power)

d = 9 (effect size)

The calculated sample size was 5 per group. To compensate for potential sampling loss, an additional 10% was included, resulting in a final sample size of 10 samples per group.

Collection and Authentication of Plant Material

Fresh, ripe, and healthy fruit samples of orange (Citrus sinensis), jackfruit (Artocarpus heterophyllus), pineapple (Ananas comosus), watery rose apple (Syzygium aqueum), and ice apple (Borassus flabellifer) were procured from local markets in Dakshina Kannada. The samples were authenticated by a qualified botanist,

and voucher specimens were preserved in the herbarium of a recognized institution.

The fruits were thoroughly washed with distilled water, peeled, and the pulp was separated and stored under refrigerated conditions until further processing.

Preparation of Fruit Extracts

The preparation of fruit extracts was carried out as follows:

1. Washing and Drying: The edible portions of the fruits were washed thoroughly and shade-dried at room temperature for 7–10 days until complete drying was achieved.

2. Grinding: The dried material was pulverized into a fine powder using a mechanical grinder.

3. Solvent Extraction:

- Twenty grams of powdered sample was soaked in 200 mL of 95% ethanol in sterile conical flasks.
- The mixtures were placed on a shaker at 150 rpm for 48 hours at room temperature.
- The extracts were filtered using Whatman No. 1 filter paper.
- The filtrates were concentrated to dryness under reduced pressure using a rotary evaporator.
- The dried extracts were weighed, stored in sterile airtight containers, and maintained at 4°C until use.

4. Preparation of Stock Solution: Each dried extract was reconstituted in dimethyl sulfoxide (DMSO) to obtain a stock concentration of 100 mg/mL.

Study Groups

The samples were divided into six groups:

Group 1: Nystatin (positive control)

Group 2: Orange pulp extract (Citrus sinensis)

Group 3: Jackfruit pulp extract (Artocarpus heterophyllus)

Group 4: Pineapple pulp extract (Ananas comosus)

Group 5: Watery rose apple pulp extract (Syzygium aqueum)

Group 6: Ice apple pulp extract (Borassus flabellifer)

Preparation of Standard Drug

Nystatin was used as the positive control at a concentration of 100 IU/mL.

Test Organism

A standard strain of Candida albicans (ATCC 10231) was used. The organism was revived and maintained on Sabouraud Dextrose Agar (SDA) slants at 4°C.

Minimum Inhibitory Concentration (MIC) – Broth Microdilution Method

1. Preparation of Serial Dilutions:

Stock solutions (100 mg/mL) of each extract were serially diluted in RPMI-1640 broth to obtain concentrations ranging from 1.56 to 100 mg/mL.

2. Inoculum Preparation:

The Candida albicans suspension was adjusted to 0.5 McFarland standard and further diluted to achieve a final concentration of 1×10^3 CFU/mL.

3. Microdilution Procedure:

- 100 µL of each extract dilution was added into 96-well microtiter plates.
- 100 µL of the standardized inoculum was added to each well.
- Nystatin served as the positive control, while wells containing only broth and inoculum served as the growth control.

4. Incubation:

Plates were incubated at 37°C for 48 hours.

5. Determination of MIC:

The MIC was defined as the lowest concentration of extract showing no visible turbidity when compared to the control. Optical density was further confirmed at 530 nm using a microplate reader.

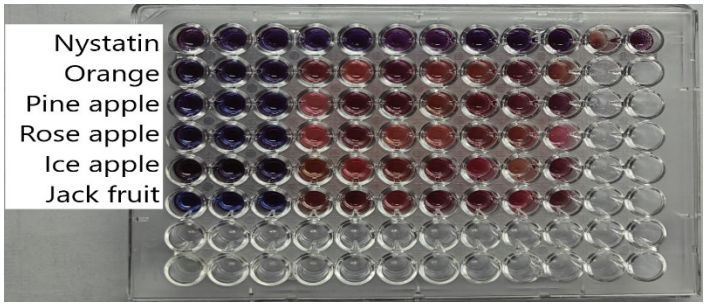

Figure 1: MIC

Zone of Inhibition (ZOI) – Agar Well Diffusion Method

1. Preparation of Inoculum:

Candida albicans suspension was adjusted to 0.5 McFarland standard (approximately 1×10^6 CFU/mL).

2. Inoculation of Plates:

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swabbed with the inoculum.

3. Well Formation:

Wells of 6 mm diameter were created using a sterile cork borer.

4. Loading of Extracts:

- 50 µL of each fruit extract (100 mg/mL) was dispensed into respective wells.
- Nystatin was used as the positive control and DMSO as the negative control.

5. Incubation:

Plates were incubated at 37°C for 24–48 hours.

6. Measurement of ZOI:

The diameter of the zone of inhibition was measured in millimeters (mm) using a digital caliper.

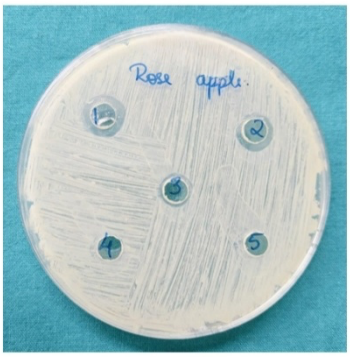

Figure 2: ZOI – Rose Apple

Figure 3: ZOI – Jack Fruit

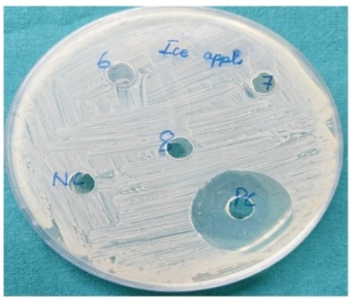

Figure 4: ZOI – Ice Apple

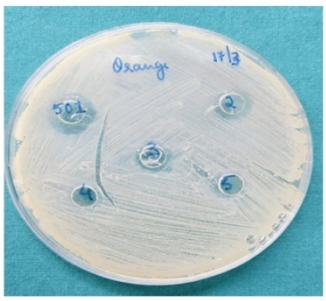

Figure 5: ZOI – Orange

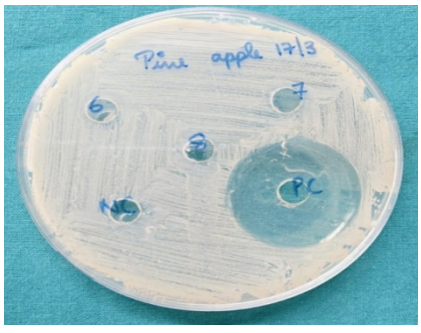

Figure 4: ZOI – Pine Apple

RESULTS

Minimum Inhibitory Concentration (MIC)

The descriptive statistics for MIC are presented in Table 1. Nystatin exhibited a mean MIC of 0.10% with no variability (SD = 0.00), indicating complete consistency across all replicates. In contrast, all five fruit extracts—Citrus sinensis, Artocarpus heterophyllus, Ananas comosus, Syzygium aqueum, and Borassus flabellifer—demonstrated identical mean MIC values of 12.50%, also with zero standard deviation, reflecting uniform and reproducible results.

Intergroup comparison using independent sample t-tests revealed that Nystatin had a significantly lower MIC compared to each fruit extract (mean difference = 12.40%, t = 34.85, $p < 0.001$). However, one-way ANOVA among the five fruit extracts showed no statistically significant difference ($F = 0.00$, $p = 1.00$), indicating comparable antifungal activity among the extracts in terms of MIC.

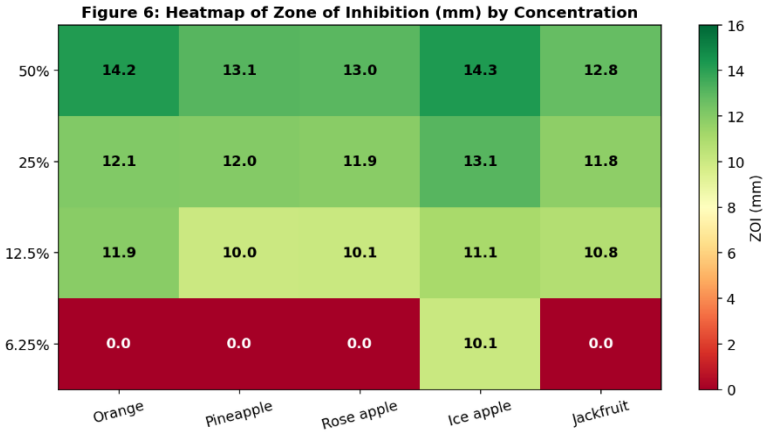

Figure 1: Zone of Inhibition (Well Diffusion)

Color-coded heatmap visualizing inhibition intensity. Darker green represents stronger activity. Ice apple shows the widest activity profile, retaining inhibition at 6.25% where all other extracts show zero activity (shown in red).

Zone of Inhibition (ZOI)

At 50% Concentration

Descriptive analysis (Table 2) showed that Nystatin produced the highest mean zone of inhibition (34.00 ± 1.20 mm). Among the fruit extracts, Borassus flabellifer (ice apple) demonstrated the largest zone (14.30 ± 0.48 mm), closely followed by Citrus sinensis (14.20 ± 0.63 mm). Ananas comosus, Syzygium aqueum, and Artocarpus heterophyllus exhibited comparatively smaller zones.

One-way ANOVA revealed a statistically significant difference among the fruit extracts ($F = 27.83$, $p <$

0.001). Tukey's post-hoc analysis demonstrated that ice apple showed significantly greater inhibition compared to pineapple, rose apple, and jackfruit ($p < 0.001$), but no significant difference was observed between ice apple and orange ($p = 0.98$). Comparisons among pineapple, rose apple, and jackfruit were not statistically significant.

Independent t-tests confirmed that Nystatin produced significantly larger zones of inhibition than all fruit extracts ($p < 0.001$).

At 25% Concentration

At 25% concentration all fruit extracts exhibited measurable antifungal activity. Ice apple showed the highest mean ZOI (13.10 ± 0.57 mm), followed by orange, pineapple, rose apple, and jackfruit, with minimal variability across replicates.

At 12.50% Concentration (MIC Level)

At the MIC level all extracts continued to demonstrate measurable inhibition. Orange showed the largest mean ZOI (11.90 ± 0.57 mm), followed by ice apple, jackfruit, rose apple, and pineapple. Zones ranged approximately between 10–12 mm, indicating retained activity even at the MIC threshold.

At 6.25% Concentration

At the lowest concentration, only ice apple exhibited antifungal activity (10.10 ± 0.57 mm), whereas all other extracts showed no inhibition (0.00 mm). Independent t-tests (Table 10) confirmed that ice apple demonstrated significantly greater activity than all other extracts ($t = 34.85$, $p < 0.001$).

Test Compound	N	Mean MIC (%)	Standard Deviation (SD)	Minimum MIC (%)	Maximum MIC (%)
Nystatin	10	0.10	0.00	0.10	0.10
Orange	10	12.50	0.00	12.50	12.50
Pineapple	10	12.50	0.00	12.50	12.50
Rose apple	10	12.50	0.00	12.50	12.50
Ice apple	10	12.50	0.00	12.50	12.50
Jackfruit	10	12.50	0.00	12.50	12.50

Table 1: Minimum Inhibitory Concentration (MIC)

Overall Findings

Although all five fruit extracts exhibited identical MIC values (12.50%) with no statistically significant differences, their performance in the agar diffusion assay varied significantly. Ice apple consistently demonstrated superior antifungal activity at lower concentrations, particularly at 6.25%, where it was the only extract to retain activity. However, Nystatin remained significantly more potent than all fruit extracts across all comparisons.

DISCUSSION

The present in vitro study evaluated and compared the antifungal efficacy of selected fruit pulp extracts against Candida albicans using both broth microdilution and agar well diffusion methods. Oral candidiasis is a common opportunistic infection, particularly in immunocompromised individuals, and remains a significant clinical concern due to increasing resistance to conventional antifungal agents and recurrence (1,2,11).

In the present study, all five fruit extracts demonstrated an identical MIC of 12.50%, whereas Nystatin exhibited a significantly lower MIC of 0.10%, indicating superior anti-fungal efficacy. This is in accordance with established literature, as Nystatin acts by binding to ergosterol in the fungal cell membrane, leading to pore formation and cell death (3,4). Previous studies have consistently demonstrated the high efficacy of polyene antifungals against Candida albicans, reinforcing their role as first-line agents (11).

Interestingly, no statistically significant difference was observed among the fruit extracts in terms of MIC values. This suggests comparable antifungal potential under both conditions, likely due to the presence of

common phytoconstituents such as phenols, flavonoids, and tannins, which are known to disrupt microbial cell walls and metabolic pathways (5,12). Similar findings have been reported in studies evaluating plant extracts, where crude extracts often demonstrate uniform MIC values due to overlapping phytochemical profiles (12).

However, the agar diffusion assay revealed significant differences among the extracts, particularly at higher concentrations. At 50%, Borassus flabellifer and Citrus sinensis demonstrated the highest zones of inhibition. Citrus species are known to contain bioactive compounds such as limonene, flavonoids, and citric acid, which exhibit antimicrobial effects by altering membrane permeability and enzyme activity (6,13). The notable activity of Borassus flabellifer may be attributed to its rich content of polyphenols and bioactive compounds, which have been reported to possess antimicrobial and antioxidant properties (10).

At 25% and 12.50% concentrations, all extracts retained measurable antifungal activity, demonstrating a dose-dependent response. Pineapple (Ananas comosus) contains bromelain, which has been shown to exhibit antimicrobial and anti-inflammatory properties, possibly contributing to its antifungal activity (8). Similarly, jackfruit (Artocarpus heterophyllus) contains flavonoids and lectins with known antimicrobial effects (7). The activity of Syzygium aqueum may be attributed to its phenolic compounds and antioxidant activity, as reported in previous studies (9).

A key finding of this study was that at the lowest concentration (6.25%), only Borassus flabellifer demonstrated antifungal activity, whereas all other extracts showed no inhibition. This suggests superior efficacy of ice apple at sub-MIC levels, possibly due to better diffusion characteristics or the presence of more potent bioactive compounds. This discrepancy between MIC and ZOI findings has been widely reported, as broth microdilution evaluates inhibitory concentration in a liquid medium, while agar diffusion depends on compound diffusibility, molecular size, and solubility (5,12).

Despite the observed antifungal activity of fruit extracts, Nystatin consistently demonstrated significantly larger zones of inhibition across all comparisons. This highlights the superior potency of conventional anti-fungal agents, which remain the gold standard in clinical management of candidiasis (3,11). However, increasing antifungal resistance and adverse effects associated with synthetic drugs have led to growing interest in plant-based alternatives (11,14). Natural extracts offer advantages such as biocompatibility, reduced toxicity, and cost-effectiveness, making them promising adjuncts in antifungal therapy (5,14).

The findings of this study are consistent with previous research demonstrating that plant extracts possess antimicrobial activity but generally require higher concentrations compared to standard drugs (12,13). Among the tested extracts, Borassus flabellifer showed superior performance, particularly at lower concentrations, indicating its potential as a promising antifungal agent. Further research is needed to isolate and characterize its active constituents and to evaluate their mechanisms of action.

However, this study has certain limitations. Being an in vitro study, the results may not fully replicate in vivo conditions, where host factors such as saliva, immune response, and biofilm formation play a significant role (1,2). Additionally, the use of crude extracts may have masked the activity of specific phytochemicals. Future studies should focus on purified compounds, advanced extraction techniques, and clinical trials to validate these findings.

CONCLUSION

Within the limitations of this in vitro study, all five fruit pulp extracts demonstrated antifungal activity against Candida albicans, with a uniform minimum inhibitory concentration (MIC) of 12.50%. However, significant differences were observed in the agar diffusion assay, indicating variation in antifungal efficacy among the extracts. Borassus flabellifer (ice apple) exhibited comparatively superior activity, particularly at lower concentrations, where it was the only extract to retain inhibitory potential.

Despite these findings, Nystatin showed significantly greater antifungal efficacy across all parameters, confirming its superiority as a standard antifungal agent. The results suggest that while fruit extracts possess promising antifungal properties, their clinical applicability may be limited without further refinement.

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